Experimental Ulcerative Colitis Produced by Anticolon Sera *

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THE POSSIBILITY that auto-antibodies may be responsible for the destruction of normal tissue has recently received considerable attention from a number of different investigators working with different organ tissues. Some diseases such as rheumatic fever, have long been suspected of being allergic in nature and the presence of circulating antibodies was suspected to have altered the host tissue response. In 1918, Guyer and Smith produced defects in the rabbit lens by injecting the rabbits with antilens immune serum.[5] This antilens immune serum was prepared by injecting minced rabbit lens into fowls. The fowls formed antibodies to the rabbit lens. The fowl antisera was then used to challenge pregnant rabbits. Lens defects developed in the offspring. Masugi [9] demonstrated that a disease similar to glomerulonephritis could be induced in animals with injections of antikidney sera. Masugi injected pulped dogs' kidneys into rabbits and challenged other dogs with the rabbit antisera. Even though the disease which resulted was strikingly similar to human glomerulonephritis, the experimental disease was considered to be a pathological curiosity unrelated in any way to the human disease.

Interest in the pathologically-induced disease was resurrected when it became possible to transplant kidneys from one identical twin to another. In several cases kidney transplants were made from a normal identical twin to the other identical twin in the late stages of glomerulonephritis. Although skin grafts take in identical twins, the kidneys thus transplanted develop glomerulonephritis.[10] It was apparent that antikidney antibody was circulating in these individuals at a time when the disease was considered quiescent and inactive.

Recently, Thal has shown that the destruction of pancreatic tissue in the experimental animal results in the appearance of immune antibodies to pancreas.[12] Antibodies to skin also appear after destruction of skin following a severe burn.[1] Antibodies to kidney can appear after injection of rats with kidney extracts.[7] Antibodies to thyroid have been demonstrated in normal humans in cases of thyroiditis.[8] Injection of pooled guinea pigs adrenal suspension will induce antibody formation to the adrenal gland.[13] It is thus apparent that antibodies to organ tissue can develop and that the stimulus for this development may often be the necrosis of the organ tissue in question. More recently, Broberger had demonstrated the presence of auto-antibodies to colon in human cases of ulcerative colitis.[2]

Except for Masugi's demonstration that a disease could be produced by these antibodies, the sole demonstration of autoantibodies is not sufficient proof that they play any causal role in the development of the disease. To prove that antibodies produce colonic disease in the animal, an attempt was made to induce ulcerative colitis in dogs by the injection of immune antibodies.

Materials and Method

The antigen was prepared from dog colon which had been previously rendered sterile by suitable antibiotic administration. The colonic mucosa was removed and homogenized in a Waring blender. The homogenate was strained through cheese cloth to remove large particles and penicillin and
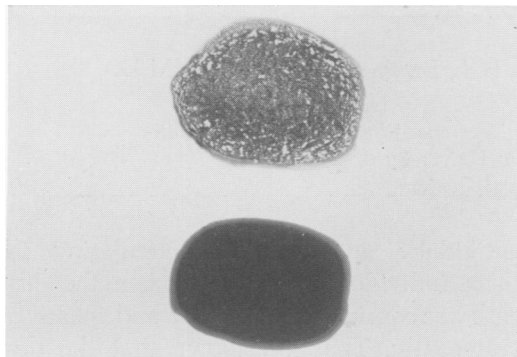
FIG. 1. A positive tanned red cell agglutination test is shown. The cells below do not agglutinate and the test is negative.

streptomycin were added as preservatives. Antibodies were produced by intraperitoneal injection of 40 per cent antigen suspension into rabbits or ducks. The animals were injected several times in the first two weeks and then an amnestic response was elicited by another injection of antigen several weeks later. No adjuvant was used since this method produced a satisfactory titer of antibodies in rabbits and ducks.

The antisera and the recipients' serum before and after the administration of the antisera were tested by the tanned red cell agglutination procedure. Red cells were coated with the antigen to sensitize them in the following manner: 2.0 cc. of 40 per cent tissue homogenate was pipetted into a test tube to which 1.0 cc. of washed human type "O" red cells was added. Then 0.5 cc. of freshly prepared ferric chloride (a 1 : 50 dilution of a tenth molar solution) was added to the homogenate cell mixture and the test tube was vigorously shaken. The tube was then incubated at 37° C. for 10 minutes. After incubation the cells were washed rapidly with saline. The antisera to be tested was added to a five per cent suspension of the sensitized red cells on a slide which was warmed and viewed in an appropriate view box (Fig. 1). This procedure was done for dogs and a similar procedure was performed for guinea pigs. A precipitent ring test was also employed in testing the sera for antibodies.

Results

Six dogs were injected with anticolon antibodies. Two with rabbit antisera and four with duck antisera. At first it was decided to give the dogs only one injection of antibodies lest they develop antibodies to the antisera with a subsequent anaphylactic response. Shortly after the intravenous injection of antisera, the dogs developed diarrhea which became bloody after one or two days. In order to increase the ratio of injected antisera to host antigen, hemicolectomies were done in a few dogs. Alteration of this ratio has been found to influence the severity of induced glomerulonephritis.[9]

The protocols of the six dogs are given below.

Dog 217: The dog was injected with 10 ml. of rabbit antisera. The animal passed loose watery stools two hours after injection. The stools continued to be loose and watery for four days after the initial injection. Proctoscopic examination of the dog revealed only a deeply congested mucosa without any visible area of frank ulceration. At this time colonic biopsy was taken under general
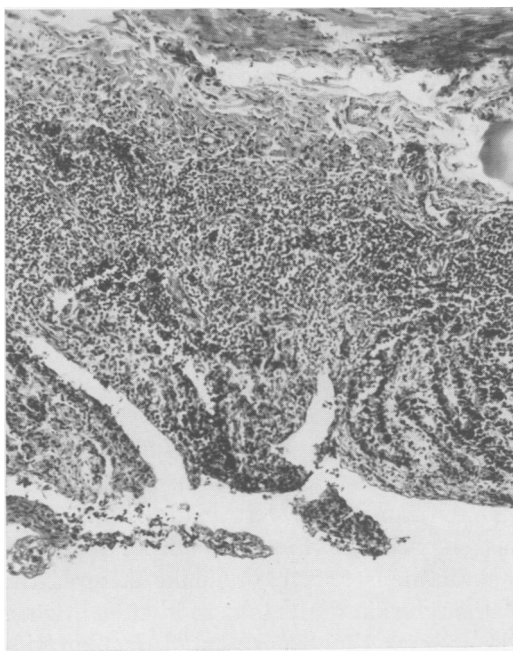

FIG. 2. A biopsy of the sigmoid four days after challenge shows acute congestion and infiltration with inflammatory cells. Ulceration of the mucosa is seen. The tips of epithelial folds are necrotic in many areas.

anesthesia. The biopsy is shown in Figure 2. The microscopic section revealed superficial necrosis of the tips of the mucosal villi and acute inflammation and congestion. The stool continued to be loose and watery for three weeks, after which time it again became formed. The stool remained firm until six months after injection when the animal had a recurring bout of bloody diarrhea lasting for about five days. The dog's serum was tested by the tanned red cell agglutination procedure for the presence of antibodies one year after the initial challenge dose. The presence of antibodies could be demonstrated even at this late date. Since the original rabbit antisera would have been completely metabolized in this length of time, the antibodies present must have been formed by the host tissues themselves. Two years after the original challenge, the tanned red cell agglutination test became negative and the dog has remained well.

Dog 221: A colonic biopsy was performed prior to the challenge with anticolon sera. The colonic mucosa in this biopsy was normal. After the dog was challenged with intravenous injection of 10 ml. of rabbit antiserum, it passed a loose watery stool within a few hours. Diarrhea continued for 12 days, becoming bloody after the third day. One month after the original challenge, the diarrhea subsided for a two-week period only to recur with a subsequent bout of bloody diarrhea which lasted one week. The dog remained well for a four-month interval thereafter, although there were several episodes of loose watery stools. Six months after initial injection, the dog was again challenged with rabbit antisera after a prophylactic dose of chlortrimeton had been given. A bloody diarrhea immediately followed with severe dehydration. The dog expired on the second day after the rabbit challenge. At autopsy the colon showed numerous punctate areas of ulceration approximately 1.0 cm. in length (Fig. 3, 4). The area between these ulcerations was deeply congested and edematous. A typical section of ulceration was taken from necropsy (Fig. 5).

Dog 276: A partial colectomy was performed, at which time 22 cm. of colon weighing 35 Gm. were removed. The normal colonic mucosa of this surgical specimen is shown in Figure 6. Three months after an uneventful recovery, the animal was challenged with 6.0 ml. of duck antiserum. Stools became soft on the second day with a trace of blood as measured by the guaiac test. On the third day the stools became watery and more strongly positive for blood. By the end of the fourth day the stools were frankly bloody. This continued for 10 days, after which time the bleeding tapered off. The dog was sacrificed 10 days
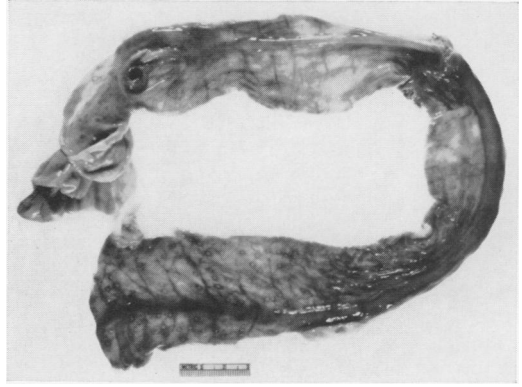

Fig. 3. The gross specimen of the colon is shown. Small areas of ulceration are scattered widely throughout the distal colon. The entire colon is congested and edematous.
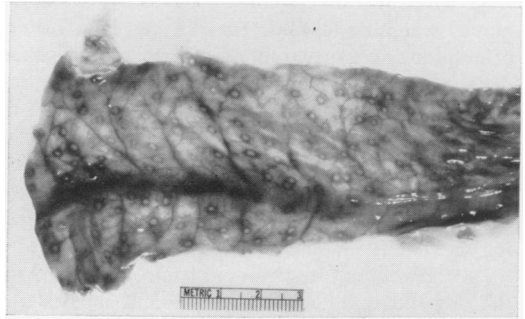

Fig. 4. Close-up of the distal colon. Numerous 3-mm. rounded areas of ulceration are seen.
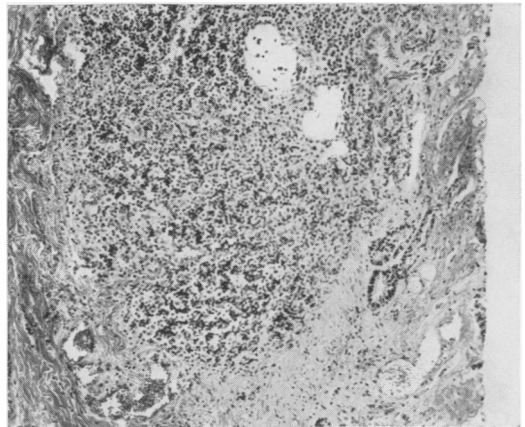

Fig. 5. Complete loss of colonic mucosa and extensive infiltration with inflammatory cells. Necrotic mucosa is visible in a few places.

post injection, at which time a colon was seen to be diffusely congested but no definite areas of ulceration were seen which would account for bloody diarrhea. Microscopy is shown in Figure 7.
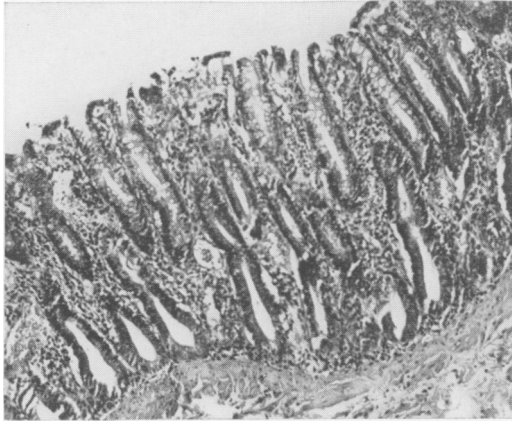
FIG. 6. The normal colonic mucosa of the removed colon is shown.

Dog 300: Fifteen cm. of the colon were removed weighing 33 Gm. The dog received 7.0 cc. of antiduck serum two months postoperatively. He developed loose watery stool four days after injection lasting for one week. A surgical biopsy at this time revealed acute inflammatory changes with necrosis of the tips of the epithelial tufts. There was no blood in the stool. Three weeks after the initial challenge the dog serum was positive for circulating anticolon antibodies.

After recovery from the post injection diarrhea, the dog did well for six weeks except for a slight weight loss. He suddenly developed severe diarrhea two months after initial injection. The diarrhea became grossly bloody on the same day. This persisted for two weeks and the dog became quite weak and emaciated. For the last two days the dog was passing frank blood. He expired 10 weeks after the initial injection of anticolon sera. At autopsy the positive findings were limited to the colon. The colon was deeply congested. There was numerous 1-mm. discrete rounded areas of ulceration scattered widely throughout the colon (Fig. 8). The microscopy showed deep congestion with minimal changes in mucosa other than the small areas of superficial ulceration.

Dog 290: A partial colectomy was done on this dog, at which time 20 cm. of colon were removed. Five weeks following the bowel resection, the stools were well formed and guaiac-negative. The dog was challenged with 7.0 ml. of duck antisera. Immediately following injection, the dog had loose watery bowel movements. This was guaiac-negative but by the end of the third day traces of blood were present. The diarrhea continued for one month with stool guaiac tests ranging from 1 to 4 +. No frank blood was seen in the stools during this period. One month following the initial challenge, the tanned red cell agglutination test showed a high titer of anticolon sera. The antisera did not react with any other dog antigen except

colon. The dog had remained well for one year. A tanned red cell agglutination test at that time was negative. The dog was sacrificed and showed a normal appearing colon.

Dog 291: Eighteen cm. of colon were removed. The dog had a stormy postoperative recovery and was not ready for injection until three months following the colectomy. Seven cc. of duck antisera were given, following which the dog developed diarrhea without frank blood. Circulating anticolon antibodies could be demonstrated by the agglutination test two weeks after the challenge. This dog is alive and is being followed. Four months after the challenge, the dog was well and the tanned red cell agglutination test was weakly positive. The dog remained well for nine months. The tanned red cell agglutination test at that time was negative.

Control Animals: Four dogs were chosen at random and were given normal duck serum or rabbit serum. These animals did not develop diarrhea and the agglutination test for anticolon antibodies was negative.

The antisera which were injected into the animals were tested for specificity with various types of antigen. The antiduck sera agglutinated with antigen from tissue of dog origin regardless of its source. In other words, the antisera in addition to being anticolon sera were also antidog sera. Immediately following injection into the dog, however, this animal's circulating antibodies showed specificity for colon. It is possible that the weak antidog titer was rapidly neutralized by the host tissue. The titers for colonic tissue were much higher than for other tissues. Other specimens of the gastro-intestinal tract also gave a weak response with canine serum showing antibodies showed cross immunity with other intestinal anti-
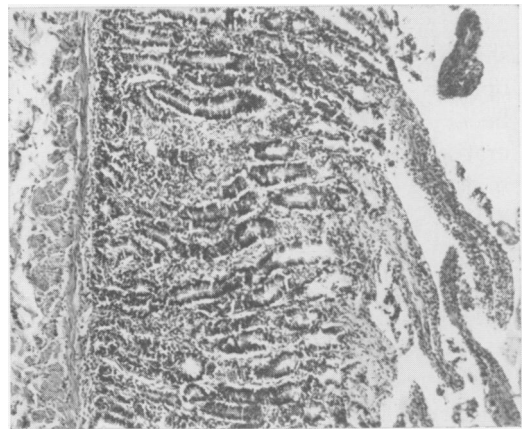

FIG. 7. The acutely congested bowel following challenge. Mucosal tips are necrotic and the surface of the mucosa is covered by an inflammatory exudate. Submucosa is infiltrated with inflammatory cells.

gen. This does not mean that antibodies for colon epithelium were not specific, but that these antibodies can react weakly with similar antigens.

All attempts at production of a pathologic lesion in the guinea pig have yielded negative results. Challenging the guinea pig with anti-guinea pig colon has led only to mild edema and congestion of the mucosa with no colonic symptomatology such as diarrhea.

Discussion

The demonstration of circulating antibodies to colon is not sufficient to prove that human ulcerative colitis is caused by an auto-immune mechanism. In order to fulfill Koch's postulates, it is necessary to induce the disease in experimental animals by the injection of antibodies. When dog's colon is used as an antigen, antisera can be formed in experimental animals and the re-injection of this antisera is capable of inducing an ulcerating lesion not unlike the acute phases of ulcerative colitis. The induced disease is subject to remissions and exacerbations similar to that seen in human ulcerative colitis. Chronic lesions have not been produced, but this may be due to the short duration of our experiments and also to a possible species difference. In our long term experiments, the animals escaped from the continued production of antibodies. Continuous destruction of tissue did not occur, therefore antigen did not gain access into the circulating blood to repeatedly stimulate antibody formation. The continued production of antibody should be a prerequisite for the development of a chronic disease. There has been adequate demonstration in the literature that antibodies can form when homologous dead tissue is injected into an animal or when tissue destruction has caused necrosis of organ tissue. The sympathetic ophthalmia which develops in the normal eye after injury of the other has long been considered an autoimmune response. In some cases positive skin tests have been elicited with extracts of eye tissue in patients developing sympathetic ophthalmia. However, that such antibodies can be destructive to normal tissue is not generally appreciated. The important link has been
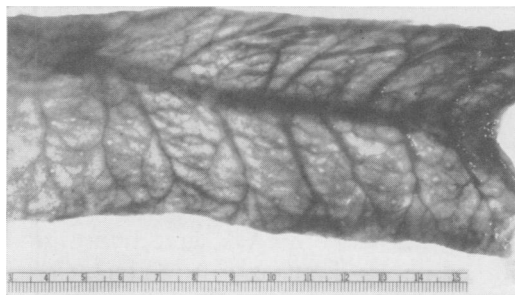

FIG. 8. Gross autopsy appearance of the large bowel on Dog 300. Notice intense vascular congestion and small punctate areas of ulceration.

the demonstration that antibodies can destroy normal tissue and thereby stimulate the further production of similar antibodies. Our experiments have closed this gap.

Our experimental work suggests the following pathogenesis for ulcerative colitis. Infection, ischemia, or trauma, cause destruction of colonic tissue. Colonic protein gains access into the circulation and antibodies are formed against the colonic tissue. These antibodies cause further destruction of remaining colon tissue, inducing a vicious circle. The experiments also suggest a wide difference in response of the host. Our attempts to produce a lesion in guinea pigs by a technic identical to that used in the dogs was a failure. This may be similar to what has been described in the experimental production of glomerulonephritis. In this disease the severity of the induced disease is apparently related to the ratio of antigen (normal colon) to the amount of anticolon injected. The severity of the induced lesion is therefore enhanced by increasing the amount of antiserum. Aside from this quantitative difference, there are also individual differences in resistance to disease and development of antibodies. All dogs do not show the same severity of disease with the same dose of antisera.

One factor remains mysterious. If one subscribes to the auto-immune mechanism for production of ulcerative colitis, can one account for the occasional familial history which is found in some patients with auto-immune diseases such as rheumatic fever. This familial history is also occasionally

seen in ulcerative colitis. The experimental work of Guyer and Smith [6] offers an interesting explanation for the transmission of auto-immune disease. These authors injected pulped rabbit's lens into fowls. When the fowl antisera is administered to pregnant rabbits, lens defects developed in the young, but these defects were transmitted through male line progeny and appeared as Mendelian recessive characteristics. Our failure to produce ulcerative colitis in the guinea pig precluded the possibility of testing this interesting hypothesis for ulcerative colitis.

Burnet [3, 4] has offered an interesting theory which also might explain the occasional familial history of ulcerative colitis. Antibody production by immune cells is controlled by the genes, actually the desoxyribonucleic acid of the cell. Each immune cell produces only a few different antibodies. The original cell and its offspring (called clones) continue to produce the same antibodies. Antigen stimulates the reproduction of a particular clone which produces antibody to it although an overwhelming dose of antigen can produce immune paralysis. The survival of clones follows the theory of natural selection as proposed by Darwin. Useful clone variants survive while harmful clone variants do not survive either because the immune cell is destroyed, or the entire animal may be destroyed. In runts, a forbidden group of clones produces fatal antibodies against itself. The clonal selection hypothesis offers a satisfactory explanation for the familial tendency in the auto-immune diseases. Actually, the destruction of tissue floods the host with antigen and allows for the continued propagation of forbidden clones. The clones produce more antibody which lead to further tissue destruction. A vicious circle is thus established. The genetic capacity for antibody production is transmitted to offspring. Immunity to some infectious diseases, such as tuberculosis, has been shown to be a heritable characteristic. These studies done on twins suggest similar studies for ulcerative colitis.

The auto-immune mechanism may not only be responsible for the production of ulcerative colitis, but may be responsible for the development of such diseases as cirrhosis, pancreatitis, prostatitis, nephritis, thyroiditis, and similar diseases. The production of pancreatitis and cirrhosis has now been undertaken in our laboratory by auto-immune methods. This study suggests the possibility that many nonspecific inflammatory diseases of organs may be caused by auto-immune mechanisms.

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